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The Effect of Different Harvest Strategies on the Nucleated Cell Yields of Bone Marrow Collection

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To improve bone marrow (BM) harvest of the volunteer donors in our institute, we changed from the single-hole needle to the multi–side-hole needle after March 2002, and examined the midway total nucleated cell (TNC) counts during collection after September 2004. The aims of this retrospective study were to evaluate BM harvest yields obtained through different strategies and to examine the correlation between final and midway BM harvests. The distribution of BM harvesting by different strategies was 235 donors with single-hole needles (group A), 389 donors with 5–side-hole needles (group B), and 365 donors with 5–side-hole needles and midway TNC counts (group C). The nucleated cell density of the collected BM was significantly improved by modifying the harvest strategy (0.202×10^8/mL in group A, 0.219×10^8/mL in group B, and 0.250×10^8/mL in group C; $P < .001$). The percentage of unacceptable TNC dose ($<2 \times 10^8$/kg) was also decreased in all 3 groups (to 5.9%, 3.6%, and 0%, respectively; $P < .001$). Multiple regression analysis revealed that donor weight, white blood cell count, and harvest strategy were positively correlated with BM TNC density ($P < .001$), whereas harvested BM volume was negatively correlated with TNC density ($P < .001$). On linear regression analysis, highly significant correlations were noted between midway and final TNC densities ($r = 0.8774$; $P < .001$) as well as between harvested BM volume and TNC count ($r = 0.7937$; $P < .001$). Changing the harvesting needle and checking the midway TNC count improved the harvest outcome.

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INTRODUCTION

Over the past several decades, bone marrow transplantation (BMT) has been used to successfully treat numerous malignant and nonmalignant hematologic diseases. After HLA matching, adequate BM cell dose is one of the most important donor factors in successful hematopoietic stem cell (HSC) transplantation. The common criterion of acceptable cell dose is $3\text{-}5 \times 10^8$ of nucleated cells per kg of recipient body weight. A higher total nucleated cell (TNC) count will improve patient outcomes [1-4]. An unacceptable cell dose has been recommended as the nucleated cells were $<2 \times 10^8$/kg [5], but it is difficult to predict final harvest yield before this procedure. We have previously demonstrated that donor body weight and baseline white blood cell (WBC) count are positively correlated with the cell density of the collected BM, whereas harvested BM volume is negatively correlated with cell density [6]. None of these donor factors except BM harvest volume is modifiable, however.

It is important to minimize the side effects of the BM harvesting procedure on the donor and also to have a sufficient volume of collected BM to ensure a better recipient outcome. Few previous studies have addressed strategies to improve the harvest yield of BM collection. Most of these focused on the amount of aspiration at each puncture site or the use of granulocyte colony-stimulating factor (G-CSF) as priming [7-11]. Less aspiration per puncture might increase the TNC count by avoiding contamination of peripheral blood (PB), but this strategy will increase the number of punctures and prolong the duration of anesthesia, both of which are strongly associated with BM harvest complications [12]. Higher total collected BM volume

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also might be associated with increases in other post-collection symptoms, such as those of anemia.

The Tzu-Chi Marrow Donor Registry was established in 1993 and was renamed the Buddhist Tzu-Chi Stem Cell Center (BTCSCC) in 2002. There are currently 330,000 enrolled donors, with >2000 stem cell collections (1136 BM and 940 PB stem cell [PBSC]) performed as of December 2009. The BTCSCC is one of the most active HSC registries in Asia, with >50% of the HSC donations destined for patients overseas. To improve the BM harvest outcome in our institute, we changed some of our harvest strategies in recent years. First, we changed from a single-hole needle to a multi–side-hole needle in March 2002. The harvest needle that we currently use has 5 side holes in addition to the tip hole and can aspirate 6 separate BM compartments simultaneously. Theoretically, use of the multi–side-hole needle can reduce the harvest time and minimize the contamination of PB. Meanwhile, the shorter operation time and anesthesia also might decrease BM harvest complications for the donors. Second, to avoid unacceptable nucleated cell doses for transplantation, in September 2004, we started to check the TNC count midway during BM collection in the main harvest center, Tzu-Chi General Hospital in Hualien. The midway TNC count might help the attending physician determine the final harvested BM volume.

The aim of this retrospective study was to evaluate the effect of the changes in harvest strategies on BM harvests in our institution. BM nucleated cell density (TNCs per mL of harvested BM) was used as the major outcome indicator of harvest yield for comparisons, to avoid bias resulting from varying recipient weight and donor harvest volumes. The correlations among TNC count, harvested BM volume, and TNC density of collected BM also were analyzed to help predict harvest outcome.

MATERIALS AND METHODS

Between January 1999 and July 2009, all healthy volunteers who underwent BM donation at the Buddhist Tzu-Chi Stem Cell Center were included in this retrospective analysis. Standard BM harvest procedures were performed by staff at the Department of Oncology and Hematology in the 2 branches of Tzu-Chi General Hospital (Hualien and Dalin). All participants agreed to BM stem cell donation after receiving explanations of the procedures for BM harvest and PBSC collection. Informed consent was obtained from each donor before the harvest procedure. The study design was approved by the hospital's Institutional Review Board.

Under general anesthesia, BM was aspirated from the bilateral posterior iliac crest using a single-hole needle. After March 2002, our institution replaced the single-hole harvest needle with a new harvest needle with 5 additional side holes. The targeted final collected BM volume was estimated by 20 mL per kg of the recipient's body weight, with a maximum amount of 20 mL per kg of the donor's body weight. To reduce the contamination of PB in the harvested BM, the volume of each aspiration was restricted to 5 mL with the single-hole needle and 30 mL with the multi–side-hole needle. A technician monitored the amount of aspiration during BM collection. After September 2004, the midway TNC count was checked at Tzu-Chi Hospital Hualien by the consensus of harvest physicians. Two mL of harvested BM was sent for TNC counting at approximately the midway point of the targeted harvest volume. The stopping point of the harvest was at the discretion of the attending physician, based on collecting 20 mL of BM per kg of recipient weight and the midway TNC count if data were available. The collected BM was mixed with citrate dextrose (ACD) at a ratio of 0.28 and filtered through 500-μm and 200-μm filters successively. The total volume of the harvested BM was obtained by subtracting the volume of anticoagulant from the volume of the mix. The midway and final nucleated cell concentrations of the harvested BM were determined using an automatic hematology analyzer (XE-2100; Sysmex, Kobe, Japan). The TNC density in the harvested BM was determined by dividing the number of TNCs by the volume of the harvested BM.

Statistical Analysis

Analysis of variance and the χ^2 test were used to determine the significance of differences between the different strategy cohorts. The TNC density of harvested BM was correlated with donor characteristics, including age, sex, body weight, and baseline white blood cell (WBC) count, and harvest strategies by multiple regression analysis. The cell densities of the midway and final harvest products were compared using the paired-sample *t* test. Linear regression analysis was used to evaluate the relationship between harvested BM volume, TNC count, and cell densities in donors in whom both midway and final TNC counts were checked. A P value $< .05$ was considered statistically significant. All analyses were done using MedCalc for Windows, version 9.6.0.0 (MedCalc Software, Mariakerke, Belgium).

RESULTS

A total of 989 volunteer donors (408 [41.3%] males; mean age, 29.6 years [range, 17-55 years]) underwent BM harvest for unrelated BM transplantation at Tzu-Chi General Hospital in Hualien and Dalin during the study period. The distribution of BM harvests

Table 1. Donor Characteristics and Results of BM Harvesting

	Group A	Group B	Group C	*P*
N	235	389	365	
M/F, %	38/62	38/62	47/53	.024
Age, years	29.4	29.1	30.3	.055
Donor BW, kg	63.9	64.6	64.2	.779
BM volume, mL	944 (232-1250)	927 (203-1473)	995 (568-1599)	<.001
Recipient BW, kg	56.1	52.1	53.9	.045
BM/recipient weight, mL/kg	18.6	20.2	20.7	.005
Baseline WBC count, $10^3/\mu L$	5.97	5.95	6.25	.020
TNC count, 10^8 cells	189 (37.2-444)	201 (26.9-475)	245 (93.8-477.5)	<.001
Cell density, 10^8/mL	0.202 (0.088-0.427)	0.219 (0.072-0.462)	0.250 (0.118-0.610)	<.001
Cell dose, 10^8/kg	3.79 (1.33-15.5)	4.46 (0.99-19.2)	5.19 (2.01-27.8)	<.001
Cell dose <2 × 10^8/kg, %	5.9%	3.6%	0%	<.001
Cell dose >5 × 10^8/kg, %	15.7%	28.3%	38.6%	<.001

BM indicates bone marrow; BW, body weight; TNC, total nucleated cell.
Data are presented as mean unless indicated otherwise. Cell density, TNC/volume × 10^8/mL of BM; cell dose, TNCs/recipient weight × 10^8/kg of recipient weight.

by different strategies was 235 donors with a single-hole needle (group A), 389 donors with a 5–side-hole needle (group B), and 365 donors with a 5–side-hole needle and a midway TNC value (group C). There were 46 donors at Tzu-Chi Hospital Dalin, all of whom were in group B. Donor characteristics and harvest outcomes of the 3 groups are summarized in Table 1. Mean age and donor body weight did not differ among the 3 groups. The recipient weight was significantly greater in group A compared with group B, but not compared with group C (56.1, 52.1, and 53.9 kg, respectively; $P = .045$). The baseline WBC count was slightly higher in group C compared with groups A and B ($5.97 \times 10^3/\mu L$, $5.95 \times 10^3/\mu L$, and $6.25 \times 10^3/\mu L$, respectively; $P = .02$). The final harvested BM volume and the BM volume per kg of recipient weight were significantly higher in group C (944 mL, 927 mL, and 995 mL [$P < .001$] and 18.6 mL, 20.2 mL, and 20.7 mL/kg [$P = 0.005$], respectively).

Changing the harvest needle and checking the midway TNC count were associated with significant increases in nucleated cell density in the harvested BM in all 3 groups (0.202×10^8/mL in group A, 0.219×10^8/mL in group B, and 0.250×10^8/mL in group C; $P < .001$). The other outcome parameters, including final TNC count and cell dose per recipient weight, were also improved in all 3 groups (189×10^8, 201×10^8, and 245×10^8 [$P < .001$] and 3.79×10^8/kg, 4.46×10^8/kg, and 5.19×10^8/kg [$P < .001$], respectively). The percentage of unacceptable TNC dose ($<2 \times 10^8$/kg) was decreased (to 5.9%, 3.6%, and 0%, respectively; $P < .001$) by modifying the harvest strategy.

The multiple regression analysis revealed that donor weight, baseline WBC count, harvest group, and harvest BM volume were statistically significantly correlated with the nucleated cell density of the harvested BM (Table 2). Donor body weight, WBC count, and harvest group were positively correlated with the cell density of the BM harvest (all *P* values were <.001), whereas the total volume of the harvested BM was negatively correlated with the cell density of the BM harvest ($P < .001$). There was a trend of correlation between donor age and cell density ($P = .059$).

Base on linear regression, a highly significant correlation was found between the harvested BM volume and the midway and final TNC counts in group C (n = 730; $r = 0.7937$; $P < .001$; Figure 1). The regression equation was TNC (y) = 40.0823 + 0.2057 × BM volume (x). The mean cell density of the final harvested BM was significantly lower than that of the midway harvested BM (0.250×10^8/mL vs 0.292×10^8/mL; $P < .001$). The midway and final BM cell densities were closely correlated (n = 365; $r = 0.8774$; $P < .001$; Figure 2). The regression equation was final cell density (y) = 0.06787 + 0.6234 × midway cell density (x).

DISCUSSION

As the number of volunteer HSC donors increases worldwide, many more patients can have multiple HLA-matched donors. A recent National Marrow Donor Program (NMDP) report noted that 56% of patients have ≥10 suitably HLA matched donors in the current NMDP files [13]. We have demonstrated that donor body weight and baseline WBC cell count

Table 2. Correlation of Cell Density of BM Harvest and Donor Characteristics on Multiple Regression Analysis

	P
Age	.059
Sex	.838
Donor weight	<.001
WBC count	<.001
Harvest strategy	<.001
BM volume	<.001

BM indicates bone marrow; WBC, white blood cell.

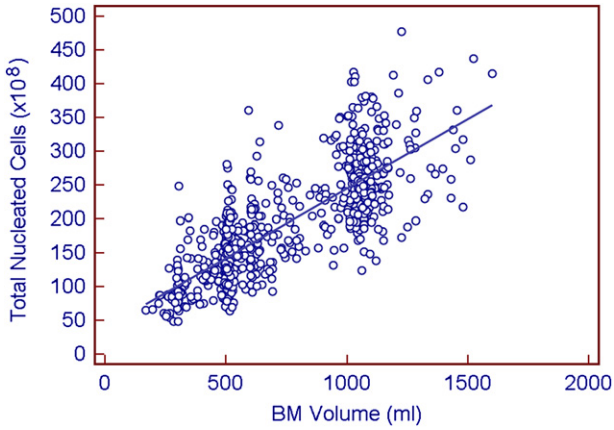

Figure 1. The correlation between TNC count and harvest BM volume in group C (n = 730; $r = 0.7937$; $P < .001$). The regression equation is TNC (y) = 40.0823 + 0.2057 × BM volume (x).

are positively correlated with the cell density of the collected BM, whereas harvest BM volume is negatively correlated with the cell density [6]. These donor factors can help guide physicians in selecting an optimal donor to provide a sufficient BM cell dose. Once the donor is chosen, the yield of the collected BM depends on the harvest procedure.

Two ways of increasing BM harvest outcome in a chosen donor are to increase the collected volume or increase the cell density of the harvested BM. The target volume of BM collection has been empirically based on the weight of the adult recipient, with a target of 15-20 mL of BM per kg recipient weight. Although this method is widely used, a significant percentage (27%-50%) of recipients receive a relatively low BM cell dose (<2.4-2.6 × 10^8/kg) [3,4]. To obtain a higher infused nucleated cell dose for transplantation, physicians usually tend to collect as high a BM volume as possible. For donors, this strategy can prolong the anesthesia time, increase the number of puncture sites, and increase blood loss. A large BM volume also might be harmful to the recipient because of the risk

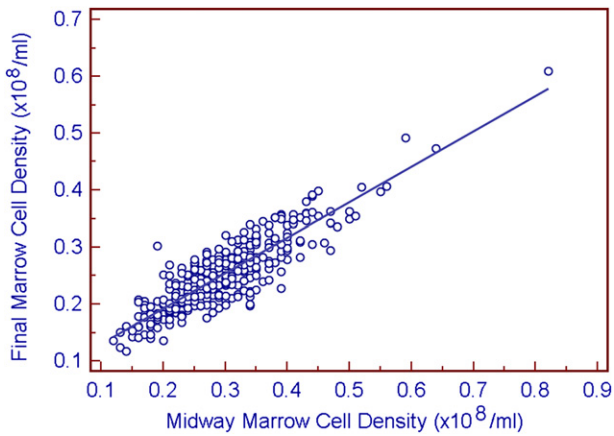

Figure 2. The correlation between midway and final BM cell density (n = 365; $r = 0.8774$; $P < .001$). The regression equation is final cell density (y) = 0.06787 + 0.6234 × midway cell density (x).

of fluid overload. The most effective and safe way to reach the target cell dose is to increase the cell density in the collected BM. In this study, we analyzed strategies aimed at improving BM harvest outcomes by increasing cell density instead of increasing the collected BM volume.

In contrast to marked advances in BM transplantation, BM harvest techniques are well established and have remained unchanged for many years except for the introduction of new harvest needles with multiple side holes. To reach the target cell dose in BM harvest, in March 2002 our institution adopted the use of a multi–side-hole aspiration needle. We restricted the amount of aspiration to 30 mL per puncture, to reduce the effect of hemodilution. Our data indicate that this new strategy led to an increased cell density of BM products and, consequently, a higher final cell dose for recipients.

In an effort to achieve even better results, in 2004, we introduced midway TNC counts. This strategy was designed to influence the practice of the collection staff. Although we did not impose a strict rule to guide the harvest based on the midway TNC count, the strategy significantly increased the cell density of the collected BM. The final collected volume was higher in group C compared with group B (995 mL vs 927 mL; $P < .001$). The increased harvest BM volume could be one reason for the increased TNC count (189 × 10^8 in group A, 201 × 10^8 in group B, and 245 × 10^8 in group C; $P < .001$), but not for the increased BM cell density (0. 202 × 10^8/mL, 0.219 × 10^8/mL, and 0.250 × 10^8/mL, respectively; $P < .001$), because of the negative correlation between collected BM volume and cell density. The influence of the midway TNC count might stem from the fact that staff were aware of the unsatisfactory TNC counts and attempted to improve harvest outcomes by further decreasing the volume of aspiration per puncture to reduce hemodilution, using new puncture locations, and increasing the final collected volume. The first two modifications might play a major role in the increased final cell density in group C by avoiding PB contamination.

Tanikawa et al. [14] first investigated the effect of the multi–side-hole aspiration needle on BM harvest. A higher mean TNC count was obtained with the multi–side-hole needle compared with the single-hole needle (33.06 × 10^3/μL vs 32.90 × 10^3/μL), but the difference is not statistically significant in this small study. Recently, Lannert et al. [15] demonstrated that replacing the single-hole harvest needle with a 5–side-hole needle significantly shortened the BM collection time, but had no effect on the cell density of the harvested BM (0.22 × 10^8/mL for the single-hole needle vs 0.18 × 10^8/mL for the multi–side-hole needle; $P = .427$). The lack of difference between the 2 types of harvest needles in that study might result from the effect of hemodilution by a large amount of

aspiration per puncture site (200 mL of BM per puncture by the multi–side-hole needle). In the present study, with the same multi–side-hole aspiration needle but a smaller aspiration amount (30 mL per puncture), we found a higher nucleated cell density (0.219×10^8/mL for group B and 0.250×10^8/mL for group C with the multi–side-hole needle) compared with Lannert et al. [15]. This comparison suggests that a small aspiration volume per puncture would improve harvest outcome, which is consistent with the results of previous studies using the single-hole needle [7,9]. Moreover, the small number of cases in the study of Lannert et al. [15] might have caused that study to have insufficient power to detect the difference.

As stated previously, the optimal final harvest volume should be balanced between the final cell yield and the potential side effects on the donor. A surrogate marker to predict final harvest outcome would be of great help in determining the final target volume. The midway TNC count during BM harvest can help collection staff properly modify the harvest procedure before it is completed. We found a highly significant correlation between the midway and final cell densities in group C ($r = 0.8774$; $P < .001$; Figure 2). These data suggest that the midway cell density can be a good predictor of the final cell density according to the regression equation. Thus, the amount of aspiration from each puncture and/or the target final BM volume could be modified to obtain an adequate cell dose with minimal iliac bone punctures. Indeed, this strategy significantly decreased the incidence of an unacceptable cell dose ($<2 \times 10^8$/kg) from 3.6% to 0% in our study.

It is difficult to explain why the baseline WBC count was slightly higher in group C compared with the other groups (5.97×10^3/μL in group A, 5.95×10^3/μL in group B, and 6.25×10^3/μL in group C; $P = .02$). The small difference in PB WBC counts might not completely explain the increases in harvested cell density and cell dose. In multiple regression analysis, the strategies of changing the harvest needle and performing midway TNC counts were significantly correlated with the final cell density of the collected BM. Donor weight, baseline WBC count, and harvest BM volume remained independent factors affecting cell density, similar to the conclusions of our previous study with fewer cases [6].

Other studies have focused on methods of increasing BM harvest yields. One study reported a reduction in BM harvest time, but no increase in cell yield, from raising the room temperature during BM collection [8]. The working conditions for the collection staff and the cost of heating limit the practicality of this strategy, however. Several studies found increased harvested cell yield and accelerated engraftment with G-CSF–stimulated BM [10,11]; however, the use of this method increases the cost of BM harvesting and also raises the concern of additional side effects.

In conclusion, this study demonstrates that use of a multi–side-hole aspiration needle and a midway TNC count are effective strategies for achieving a higher cell density of harvested BM and, consequently, a higher cell dose for transplantation. We believe that such strategies, accompanied by optimal donor selection will improve the outcome of BM transplantation.

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